FRET to examine the dynamics of the NMDA receptor specifically with respect to the cleft closure conformational change of the isolated agonist-binding domain of GluN1 when bound to ligands of varying efficacy. These studies reveal differences in the range of cleft closure states occupied by the agonist-binding domain with the antagonist DCKA-bound form and the full agonist glycine-bound form showing a large range of cleft closure states, while the partial agonists ACBC and L-alanine, as well as the full agonist D-serine, have a much narrower spread in their cleft closure states. Further analysis shows that the fractional occupancy of the isolated domain in cleft-closure states below a threshold does correlate with agonist efficacy, providing a link between agonist-binding domain dynamics, along with cleft closure, and channel activity.

**1433-Pos Board B384**
**Reduced Curvature of Ligand-Binding Domain Free Energy Surface Underlies Partial Agonism at NMDA Receptors**
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NMDA receptors are ligand-gated ion channels that mediate excitatory synaptic transmission in the central nervous system. Partial agonists elicit submaximal channel activation, but crystal structures of the ligand-binding domain (LBD) bound with partial and full agonists show little difference. To uncover the molecular mechanism for partial agonism, here we computed the free energy surfaces of the GluN1 (an obligatory subunit of NMDA receptors) LBD bound with a variety of ligands. The free energy minima are similarly positioned for full and partial agonists, but the curvatures are significantly reduced in the latter case, indicating higher probabilities for sampling conformations with a not fully closed domain cleft. The free energy surfaces for antagonists have both shifted minima and further reduced curvatures. Reduced curvature of free energy surface appears to explain well the partial agonism at NMDA receptors and may present a unique paradigm in producing graded responses for receptors in general.

**1434-Pos Board B385**
**Measurement of Nr1/Nr2B NMDA Receptor Currents on a Microfluidic Benchtop Automated Electrophysiology Platform**
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The N-methyl-D-aspartate (NMDA) receptor is a central nervous system glutamate receptor implicated in synaptic transmission and memory function. It is also a prime target in ion channel drug discovery for both academic and pharmaceutical laboratories. The NMDA receptor has interesting biophysical characteristics in that activation of the NMDA receptor requires two coincidental events; the binding of glutamate and depolarization to remove magnesium ions that block the ion conducting pore at resting membrane potentials. We present here studies performed using a microfluidic system capable of recording from 32 experimental patterns at once. Each experimental pattern is self-contained and can deliver 8 unique solutions through individual fluidic channels to the cells in the recording chamber. Multiple solutions can flow past the cells at once providing receptor activation or protection from ligand depending on the flow pattern used. These types of fluidics have proven to be useful for performing experiments to explore Positive Allosteric Modulators (PAM) on multiple fast ligand-gated channels including nicotinic receptors. Groups of 20 cells are measured simultaneously to mitigate biological variability. Each of the 32 experimental patterns records from 2 groups of 20 cells each, potentially resulting in 32 recordings performed in duplicate. Results of these experiments are measured and compared to results using commercially available automated patch clamp systems. The NMDA receptor cell line was kindly provided courtesy of ChanTest corporation (Cleveland, OH)

**1435-Pos Board B386**
**The Structural Basis of Negative Cooperativity between Subunits of the NMDA Receptor**
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NMDA receptors are crucial signaling proteins in the mammalian central nervous system and play roles in development, synaptic maturation and learning and memory. As such, the biophysical properties of these channels can profoundly shape physiological function. For example, the biophysical property of pore block by magnesium ions endows NMDA receptor with the ability to act as synaptic co-incidence detectors. Here we examine the biophysical property of negative co-operativity between the co-agonists glutamate and glycine. It has been previously reported that the glutamate bound to the GluN2A subunit speeds the dissociation of glycine from the GluN1 subunit. Using rapid perfusion methods on outside-out patches, we confirm that glycine dissociates from the GluN1 subunit roughly 3 fold faster when glutamate is bound to GluN2A then when GluN2A is unliganded. We also report that glutamate dissociates from GluN2A approximately 2 fold faster when glycine is bound compared to when GluN1 is unoccupied. We hypothesize that this co-operativity arises from long unstructured loops of amino acids, found in the upper ligand binding domain (LBD) lobes of GluN1 and GluN2, interacting between LBD dimers. To test this, we are using fluorescence labelling of unnatural amino acids and luminescence resonance energy transfer experiments to measure conformational changes between LBDs of different dimers under varying occupancy conditions. By combing these measures of conformational change with electrophysiology and mutations blocking co-operativity, we aim to elucidate the structural mechanism of negative co-operativity at NMDA receptors.

**1436-Pos Board B387**
**Simulated Closing of the NMDA Ligand-Binding Domain**
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Glutamate receptors are one of the most prevalent neuroreceptors in the central nervous system, and glutamate is the main neurotransmitter found in the body. Structurally, glutamate receptors are huge, complex, tetrameric, multi-domain proteins that possess several possible drug-binding sites throughout the different domains.
Glutamate receptors can be over stimulated by excess glutamate or excitotoxins, causing neurodegeneration and neuronal damage through excitotoxicity. Due to their role in excitotoxicity, glutamate receptors are thought to be involved in many neurogenerative diseases, such as Alzheimer's and forms of Parkinson's.
The mechanism of activation of glutamate receptors occurs when a glutamate molecule binds to the binding site, located in the center of a 'clamshell′-like ligand binding domain (LBD). Upon binding, the bottom half of the clamshell closes over the ligand. This closing movement in turn causes the top half of the transmembrane domain to also move outwards, thus opening the channel of the receptor. Once the channel is open, cations are able to flow across the membrane, thus potentiating nerve transmission.
One of the major subtypes of glutamate receptors are the AMPA receptors, named for the additional agonists (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) that bind to them with high specificity.
Using multiple molecular dynamics (MD) sampling techniques we have resolved the detailed closing mechanism of an AMPA receptor clamshell LBD after ligand binding has occurred. A combination of both multiple brute force and nudged elastic band MD methods allowed for the energetic refinement of a pathway observed in unbiased simulations.
The closing movement is revealed to be highly asymmetric and possibly stepwise in nature. This closing motion may reveal how the AMPA receptor channel gates upon ligand activation of the receptor, and indicates that there could be intermediate 'activated' states.

**1437-Pos Board B388**
**Effect of Phosphorylation on Structure of C-Terminal Segment of AMPA Receptor**
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The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is the primary contributor to fast excitatory transmission in neurons. The AMPA receptor can be divided into four domains. Extracellularly, there are the amino-terminal and ligand-binding domains. The transmembrane domain serves as the actual ion-channel pore and, of course, links the extracellular domains to the cytoplasmic domain. Of these four domains, the structure of the outermost three has been shown in detailed crystal structures of the tetramer. However, very little is known about the structure of the cytoplasmic domain. Although it is widely thought that this segment is highly disordered, it is unknown whether local order (higher levels of secondary and/or tertiary structure) exists in the cytoplasmic terminus, or whether structural changes may occur as conformational shifts in the terminal due to functional modifications. Previous studies have established phosphorylation sites at residues S818, S831, and T840 in the GluA1 subtype receptor. These studies examined a representative membrane-proximal section of the GluA1 c-terminus comprising residues 809-841 in order to consider structural changes brought about by these phosphorylation events. The peptide was examined using circular dichroism (CD) investigation, which showed a conversion to greater helix content in the phosphomimetic sample. CD

studies of the peptide in a solution of small unilamellar vesicles were conducted and showed that the increase in helical content is also present in the context of close proximity to a lipid membrane. To confirm, single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated state and in the PKC$\alpha$-phosphorylated state, in order to gauge the distance between two native cysteines in the peptide. Phosphorylation yielded a reduced distance between these cysteines, indicative of a shift to more compressed secondary structure, that is, coil to helix.

**1438-Pos Board B389**

**Characterization of a "Hotspot" in the AMPA Receptor Activation Pathway**

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Ionotropic glutamate receptors (iGluRs) facilitate the bulk of synaptic excitation in the mammalian central nervous system. Structures of full-length, AMPA-type iGluRs (AMPARs) have recently been reported in conformations thought to represent resting, pre-open, and desensitized states. However, it is uncertain what molecular interactions determine whether an agonist-bound AMPAR will favor channel opening or desensitization. We previously described how the activation of kainate-type iGluRs (KARs) is dependent upon occupancy, by sodium, of an electronegative pocket in the ligand-binding domain (LBD). Subsequently, we asked to what extent this pocket, conserved amongst iGluR subfamilies, regulates AMPAR activation. To investigate this subject we utilized electrophysiological (outside-out patch) recordings from iGluR subunits transiently expressed in HEK 293 cells, as well as molecular dynamics (MD) simulations. Unlike the KAR subunit GluK2, receptors comprised of the AMPAR subunit GluA2 did not require occupancy of the pocket by a positive charge to activate. Interestingly, a lithium ion has been detected in the pocket of recent crystal structures of the GluA2 LBD. The effect of lithium in the external recording solution was to dramatically slow the desensitization kinetics of GluA2. MD simulations supported an increased affinity of the site for lithium versus sodium, and predicted that lithium binding holds subunits closer together. Through disrupting an inter-subunit electrostatic bridge adjacent to the ''cation'' pocket, the effect of lithium was greatly attenuated. In fact, the removal of key charges at this interface produced receptors barely capable of activation, although the functional deficit was rescued by the modulator cyclothiazide or co-expression with auxiliary subunits. We propose that when electrostatic interactions at the apex of the LBD are stabilized, AMPARs are primed for activation, whereas the disruption of these interactions directs receptors to desensitized states upon agonist binding.

**1439-Pos Board B390**

**Dynamics of the Cytoplasmic Region of an AMPA-Subtype Glutamate Receptor Revealed by State Dependent FRET**

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AMPA receptors (AMPARs) are glutamate-gated ion channels, which mediate fast excitatory neurotransmission in the central nervous system. Extensive crystallographic studies of the extracellular domains and, more recently, crystal structures and single particle EM reconstructions of full-length receptors have set the framework for further investigations of receptor conformational dynamics, gating mechanism and regulation. However, intracellular regions are either truncated or not resolved in these structures. Further, the conformational transitions of the intracellular domains during receptor gating have not been investigated.

In present study, we have explored single and double fusions of cyan and yellow variants of green fluorescent protein (CFP and YFP, respectively) at intracellular sites of AMPAR to enable measurement of conformational changes using Fluorescence Resonance Energy Transfer (FRET) in live cells. The fluorescent fusions retain wild-type receptor expression and kinetic properties. Fluorescence Lifetime Imaging (FLIM) showed ligand-dependent FRET efficiency. Conformational rearrangements accompanying receptor function were measured using a Patch Clamp Fluorometry (PCF) setup on live HEK 293 cells in real time. Our results suggest that FRET efficiency is dependent on the functional state of the receptor and allosteric modulation by Cyclothiazide, an AMPA receptor desensitisation blocker. Thus the intracellular sites undergo conformational rearrangements during receptor function.

**1440-Pos Board B391**

**Partial Agonist Binding Reveals a Unique Arrangement of AMPA LBDs**

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Ionotropic glutamate receptors (iGluRs) are large tetrameric membrane proteins that transduce the chemical signal from neurotransmitters into membrane depolarization at synapses in the brain. The conformational transition induced by the association of glutamate molecules to the ligand-binding domains (LBDs) of these receptors provides the free energy that drives the opening of the transmembrane ion channel. Here, we describe the crystal structure of a GluA2 LBD tetramer in presence of the partial agonist 5-fluorowillardiine (FW) (FW sLBDs). Validation of the structure by a battery of engineered metal bridges showed that this LBD configuration corresponds to an intermediate state of receptor activation distinct from the previously publish closed-angle (CA) structure. GluA2 activation therefore, involves a combination of both intra- and inter-LBD dimer conformational transitions. The presented results provide new quantitative data supporting the idea of a dynamic LBD during activation in the context of a tetramer.

**1441-Pos Board B392**

**Structural Mechanism of Glutamate Receptor Activation and Desensitization**

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Ionotropic glutamate receptors are ligand-gated ion channels that mediate excitatory synaptic transmission in the vertebrate brain. To gain a better understanding of how structural changes gate ion flux across the membrane, we trapped rat AMPA ($\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate receptor subtypes in their major functional states and analysed the resulting structures using cryo-electron microscopy. We show that transition to the active state involves a 'corkscrew' motion of the receptor assembly, driven by closure of the ligand-binding domain. Desensitization is accompanied by disruption of the amino-terminal domain tetramer in AMPA, but not kainate, receptors with a two-fold to four-fold symmetry transition in the ligand-binding domains in both subtypes. The 7.6 Å structure of a desensitized kainate receptor shows how these changes accommodate channel closing. These findings integrate previous physiological, biochemical and structural analyses of glutamate receptors and provide a molecular explanation for key steps in receptor gating.

**1442-Pos Board B393**

**Long Timescale Simulations of Ligand Binding in Glutamate Receptors**

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Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels that mediate the majority of fast excitatory transmissions in the central nervous system. They transduce chemical information upon agonist binding into electrical information at synapses. In this study, we present long timescale simulations of both ligand binding association and dissociation events. Novel intermediate states and metastable interactions are identified using potential of mean force (PMF) calculations. Kinetics are inferred using a Markov state model (MSM).

**1443-Pos Board B394**

**Can Activation and Desensitization Properties of iGluRs Be Predicted and Understood by Studying the LBD Dimer Dynamics?**

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Ionotropic glutamate receptors (iGluRs) are vital for the function of our central nervous system (CNS), e.g. in learning and memory formation, and thus implicated in many CNS disorders. The tetrameric iGluRs contain a glutamate-gated cation channel with the extracellular ligand binding domains (LBD) forming a dimer of dimers. Subsequent to channel opening,